Name: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Date: \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_ Period: \_\_\_\_\_\_

**Cell Division Lab**

AP Biology, Ms. Ottolini

***Overview:*** *In this lab, we will be investigating the effect of caffeine on the rate of mitosis in green onions (aka scallions). We will be indirectly measuring the rate of mitosis by tracking the growth/length of the roots in a control solution (distilled water) and three different solutions of varying caffeine concentrations. We will also be directly measuring the rate of mitosis by comparing the number of cells in interphase vs. mitosis in onion roots treated in each of our four solutions. (Note: a high percentage of cells in mitosis indicates that mitosis is occurring rapidly.)*

***Hypothesis:*** There will be a statistically significant difference in the rates of mitosis in onions treated with caffeine vs. control onions.

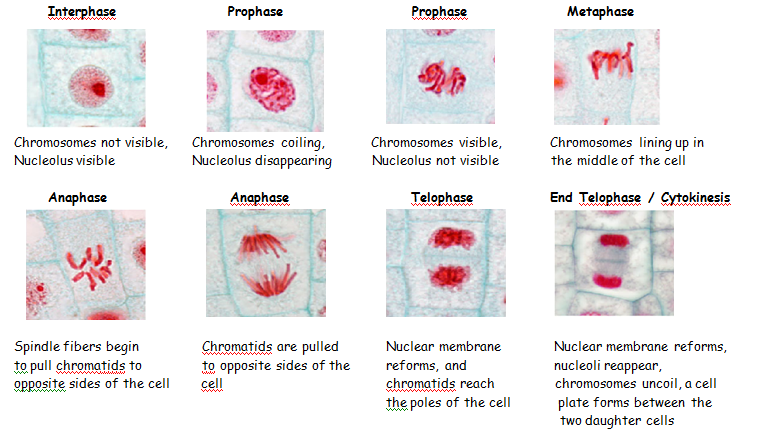
***Null Hypothesis:*** There will be no statistically significant difference in the rates of mitosis in onions treated with caffeine vs. control onions.

***Procedure – Set Up:***

1. Obtain four green onions from your teacher.
2. Trim all roots to a length of approximately 1 mm from the base of the bulb.
3. Place one onion in the class beaker of each treatment solution—distilled water, 1 mM (millimolar) caffeine, 5 mM caffeine, and 10 mM caffeine.
4. Leave the roots in the beakers for 48 hours until the next class period.

***Procedure – Collecting Data:***

1. Remove the onions from their beakers of solution.
2. Measure the length of all root tips (or just choose five if there are more than five). You are measuring new root growth, so make sure to subtract 1 mm (the initial length of the root tips) from each length.
3. Record the length of the root tips in Data Table A and determine the average/mean root length for roots from each solution.
4. Cut a 1 cm root tip from one of your onions and place it on a clean microscope slide.
5. Using a pipette, add 1 drop of 1 M hydrochloric acid to cover the root tip on the microscope slide.
6. Allow the root tip to soak in the acid for 5 minutes.
7. After 5 minutes, use a Kim wipe and carefully blot away excess hydrochloric acid from the slide.
8. Using a pipette, add 1 drop of distilled water to the root tip.
9. Use a Kim wipe to blot away excess water.
10. Using a pipette, add 1 drop of methylene blue stain to the root tip.
11. Allow the root tips to soak in the stain for 3 minutes.
12. Use a Kim wipe to blot away excess methylene blue stain.
13. Add 1 drop of distilled water to the root tip.
14. Place a cover slip on the root tip. Using the eraser end of a pencil, gently apply pressure on the cover slip to squash the root tissue. Apply an even downward pressure on the root tip and cover slip but not so hard as to break the cover slip. Do not twist or grind the cover slip.
15. Using low magnification on the microscope, focus on the root cells. Switch to medium power or high power as necessary to easily visualize the inside of the onion root cells.
16. Count the number of cells in interphase and the number of cells in mitosis. Make sure to count at least 50 cells.
17. Repeat Steps 4-16 for your other three onions from different solutions. Record your results in Data Table B.
18. See the images on the next page to clarify the difference in appearance for cells in various stages of the cell cycle. (Note: your cells will be stained blue, not pink!)



***Data Table A:***

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Solution** | **Root 1 length (in mm)** | **Root 2 length (in mm)** | **Root 3 length (in mm)** | **Root 4 length**  **(in mm)** | **Root 5 length (in mm)** | **Your Average root length (in mm)** | **Class Average Root Length (in mm)** |
| Distilled Water |  |  |  |  |  |  |  |
| 1 mM Caffeine |  |  |  |  |  |  |  |
| 5 mM Caffeine |  |  |  |  |  |  |  |
| 10 mM Caffeine |  |  |  |  |  |  |  |

***Data Table B:***

|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Solution** | **# of Your Cells in Interphase** | **# of Class Cells in Interphase** | **# of Your Cells in Mitosis** | **# of Class Cells in Mitosis** | **Class Total Cells** |
| Distilled Water |  |  |  |  |  |
| 1 mM Caffeine |  |  |  |  |  |
| 5 mM Caffeine |  |  |  |  |  |
| 10 mM Caffeine |  |  |  |  |  |

***Data Analysis Instructions:***

You will be using a Chi square test to determine if there is a statistically significant difference between the number of onion cells in interphase vs. mitosis in your control solution and one of the caffeine solutions.

**Caffeine Solution Your Teacher Has Chosen:** \_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_\_

Use your class totals to fill in the Table of Observed Values (o) given below. We will use letters to represent each of the numbers, these letters are given in the table.

**Table #1 – Table of Observed Values (o)**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Interphase** | **Mitosis** | **Total** |
| **Control** | A = \_\_\_\_\_ | B = \_\_\_\_\_ | A + B = \_\_\_\_\_ |
| **Caffeine** | C = \_\_\_\_\_ | D = \_\_\_\_\_ | C + D = \_\_\_\_\_ |
| **Total** | A + C =\_\_\_\_\_ | B + D = \_\_\_\_\_ | A + B + C + D = N = \_\_\_\_\_ |

Use the totals from Table #1 to calculate the expected values (e) using the formulas found in Table #2.

**Table #2 – Table of Expected Values (e)**

|  |  |  |
| --- | --- | --- |
|  | **Interphase** | **Mitosis** |
| **Control** | \_\_\_\_\_ | \_\_\_\_\_ |
| **Caffeine** | \_\_\_\_\_ | \_\_\_\_\_ |

Enter the observed values (o) from Table #1 and expected values (e) from Table #2 for each group into Table #3. Calculate the chi square (X2) value for the data by adding together the numbers in the right column to complete the formula:

**Table #3 – Calculation of Chi Square Value**

|  |  |  |  |
| --- | --- | --- | --- |
|  | **Observed (o)** | **Expected (e)** |  |
| **Control Interphase** |  |  |  |
| **Control Mitosis** |  |  |  |
| **Caffeine Interphase** |  |  |  |
| **Caffeine Mitosis** |  |  |  |

**Total of Right Column**  = X2 = \_\_\_\_\_

**Degrees of Freedom and Accepting or Rejecting your Null Hypothesis:**

When you have multiple independent variables (control solution vs. caffeine solution) and multiple dependent variables (cells in interphase vs. cells in mitosis), you calculate the degrees of freedom using the following formula:

*(Note: “df” stands for “degrees of freedom,” “IV” stands for “independent variables”, and “DV” stands for “dependent variables”)*

Since we have two independent variables and two dependent variables in this experiment, our df = (2-1)(2-1) = 1.

To determine whether you accept or reject your null hypothesis, look up the X2 value at one degree of freedom at p=0.05 on the table given below. This X2 value is known as the critical value.



If the X2 value that you calculated in Step 3 is higher than the critical number at the p = 0.05 level then you can reject the null hypothesis. In other words, there is a statistically significant difference between the observed (caffeine) and expected (control) results. (i.e. the observed results do not match the expected results)

If the X2 value is less than the critical number then you can accept the null hypothesis. In other words, there is no statistically significant difference between the observed (caffeine) and expected (control) results. (i.e. the observed results match the expected results)

***Analysis Questions:*** Answer the questions given below thoroughly and accurately. Your answers to these questions will help you to complete your mini lab report.

1. Does there seem to be a difference in root length (Data Table A) between your control and experimental groups? Based on the root lengths, does caffeine appear to increase or decrease the rate of mitosis in green onion roots?
2. From your data in Data Table B, does it appear that caffeine increases or decreases the % of cells in mitosis? Based on this data, does caffeine appear to increase or decrease the rate of mitosis in green onion roots?
3. Do your answers from #1 and #2 match or conflict with one another?
4. Describe your Chi square results. Do they indicate there is a statistically significant difference between the number of onion cells in interphase vs. mitosis in your control solution and one of the caffeine solutions?

**Writing Your Mini Lab Report:**

***Directions:*** You will be including a discussion /conclusion section only in this mini lab report. You mini lab report should be titled “Mini Lab Report: Cell Division Lab.” This report should be typed, 12 point font, and double spaced. Make sure to include your name, date, and class period at the top right corner of the page.

|  |  |  |  |
| --- | --- | --- | --- |
| **#** | **Requirement** | **Comments** | **Points Received** |
| 1 | You have clearly identified the purpose of this lab as well as the hypothesis and null hypothesis. |  | /2 |
| 2 | You have discussed the results from your root length measurements (citing specific numerical data). |  | /2 |
| 3 | You have drawn a logical conclusion about the effect of caffeine on onion root cell mitosis based on your root length measurements. |  | /2 |
| 4 | You have discussed the results from your interphase vs. mitosis cell count (citing specific numerical data). |  | /2 |
| 5 | You have drawn a logical conclusion about the effect of caffeine on onion root cell mitosis based on your cell count. |  | /2 |
| 6 | You have explained how your Chi square data allowed you to determine if the difference in cell counts between your control and caffeine groups was statistically significant. |  | /2 |

**Total Score: \_\_\_\_\_\_\_\_ / 12 = \_\_\_\_\_\_\_\_**